EXHIBIT AC

Polymorphism in Pharmaceutical Solids

edited by
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142. S. Duddu, and K. Weller, *J. Pharm. Sci., 85*, 345 (1996).
143. O. Funk, L. Schwabe, and K.-H. Frömming, *Drug Dev. Ind. Pharm., 20*, 1957 (1994).
144. O. Funk, L. Schwabe, and K.-H. Frömming, *Pharmazie, 48*, 745 (1993).
145. M. T. Esclusa-Diaz, J. J. Torres-Labandeira, M. Kata, and J. L. Vila-Jato, *Eur. J. Pharm. Sci., 1*, 291 (1994).
146. G. Bettinetti, A. Gazzaniga, P. Mura, F. Giordano, and M. Setti, *Drug Dev. Ind. Pharm., 18*, 39 (1992).
147. R. J. Yarwood, A. J. Phillips, and J. H. Collett, *Drug Dev. Ind. Pharm., 12*, 2157 (1986).
148. T. Oguchi, E. Yonemochi, K. Yamamoto, and Y. Nakai, *Chem. Pharm. Bull., 37*, 3088 (1989).
149. M. J. Pikal, and K. M. Dellerman, *Int. J. Pharm., 50*, 233 (1989).
150. O. Funk, L. Schwabe, and K.-H. Frömming, *J. Incl. Phen. Mol. Recogn. Chem., 16*, 299 (1993).
151. Y. Kawashima, T. Niwa, H. Takeuchi, T. Hino, Y. Itoh, and Y. Furuyama, *J. Pharm. Sci., 80*, 472 (1991).
152. Y. Fukumori, T. Fukuda, Y. Yamamoto, Y. Shigitani, Y. Hanyu, Y. Takeuchi, and N. Sato, *Chem. Pharm. Bull., 31*, 4029 (1983).
153. E. Laine, P. Kahela, R. Rajala, T. Heiklilä, K. Saamivaara, and I. Piippo, *Int. J. Pharm., 38*, 33 (1987).
154. M. Inagaki, *Chem. Pharm. Bull., 25*, 1001 (1977).
155. Y. Chikaraishi, M. Otsuka, and Y. Matsuda, *Chem. Pharm. Bull., 44*, 1614 (1996).
156. F. A. Andersen, and L. Brecevic, *Acta Chem. Scand., 45*, 1018 (1991).
157. S. P. Duddu, and D. J. W. Grant, *Thermochim. Acta, 248*, 131 (1995).
158. Y. Kim, and A. M. Haren, *Pharm. Res., 12*, 1664 (1995).
159. B. A. Hendriksen, *Int. J. Pharm., 60*, 243 (1990).

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Methods for the Characterization of Polymorphs and Solvates

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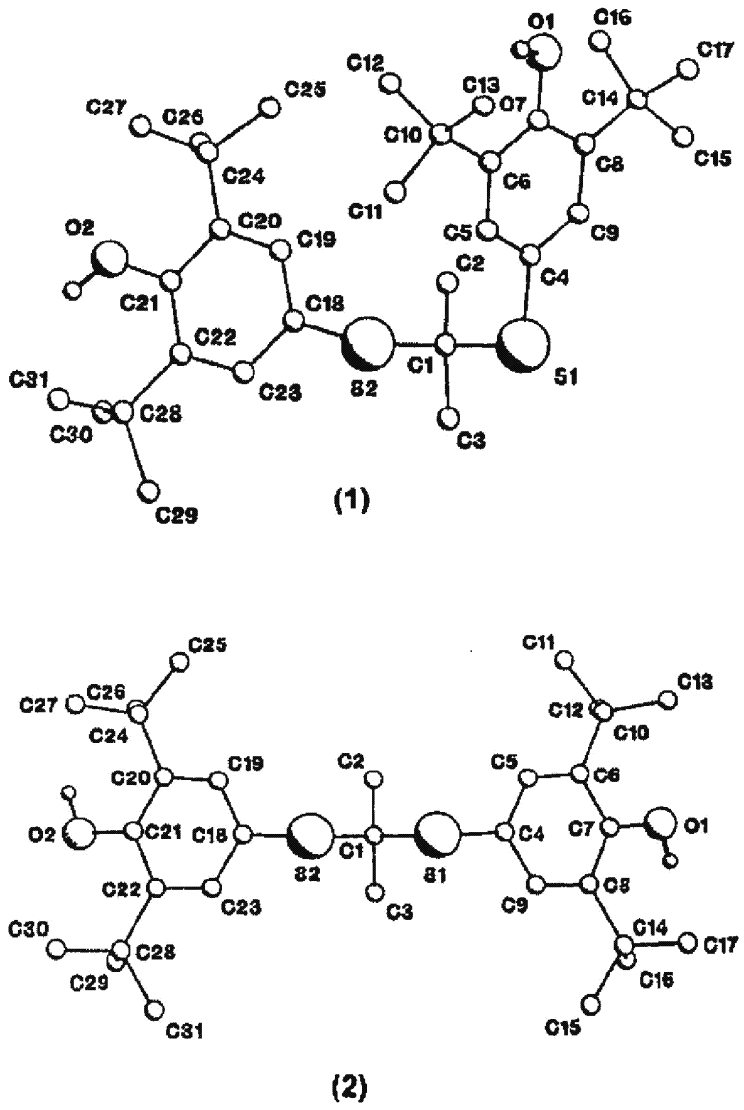

Fig. 2 Conformation of the probucol molecule existing in (1) Form I and (2) Form II. (The figure was adapted from data contained in Ref. 14).

Not all instances of conformational polymorphism are as dramatic as that just described, and often different conformers of a single side chain are able to pack into different crystalline arrangements. For instance, the two polymorphs of *p*-(1*R*,3*S*)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid were found to be associated with different conformations of the carboxylate group [15]. Torsion about a single C–N bond was shown to be the origin of the polymorphism detected for lomeridine dihydrochloride [16]. Finally, relatively small differences in molecular conformation were detected for the two polymorphic and four solvated crystalline forms of spironlactone [17].

B. X-Ray Powder Diffraction

Although the solving of a crystal structure provides the greatest understanding of polymorphic solids, the necessity for obtaining suitable single crystals and the degree of complexity associated with the data analysis preclude this technique from being used on a routine basis for batch characterization. In fact, most drug substances are obtained as microcrystalline powders, from which it is often fiendishly difficult to obtain crystallographically adequate crystals. Furthermore, during the most common evaluation of drug substances, it is usually sufficient to establish only the polymorphic identity of the solid and to verify that the isolated compound is indeed of the desired structure. For these reasons, and to its inherent simplicity of performance, the technique of x-ray powder diffraction (XRPD) is the predominant tool for the study of polycrystalline materials [18] and is eminently suited for the routine characterization of polymorphs and solvates.

A correctly prepared sample of a powdered solid will present an entirely random selection of all possible crystal faces at the powder interface, and the diffraction off this surface provides information on all possible atomic spacings in the crystal lattice. To measure a powder pattern, a randomly oriented sample is prepared so as to expose all the planes of a sample and is irradiated with monochromatic x-ray radiation. The scattering angle θ is determined by slowly rotating the sample and using a scintillation counter to measure the angle of diffracted x-rays with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed, and the detector

moved along a proscribed path to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, the spacing between the planes (identified as the d-spacings) is calculated using Bragg's law.

The XRPD pattern will therefore consist of a series of peaks detected at characteristic scattering angles. These angles, and their relative intensities, can be correlated with the computed d-spacings to provide a full crystallographic characterization of the powdered sample. After indexing all the scattered lines, it is possible to derive unit cell dimensions from the powder pattern of the substance under analysis [18]. For routine work, however, this latter analysis is not normally performed, and one typically compares the powder pattern of the analyte to that of reference materials to establish the polymorphic identity. Since every compound produces its own characteristic powder pattern owing to the unique crystallography of its structure, powder x-ray diffraction is clearly the most powerful and fundamental tool for a specification of the polymorphic identity of an analyte. The USP general chapter on x-ray diffraction states that identity is established if the scattering angles of the ten strongest reflections obtained for an analyte agree to within ±0.20 degrees with that of the reference material, and if the relative intensities of these reflections do not vary by more than 20 percent [19].

The power of XRPD as a means to establish the polymorphic identity of an analyte can be illustrated by considering the case of the anhydrate and trihydrate phases of ampicillin. The crystal structures of both phases have been obtained, and they differ in the nature of the molecular packing [20]. The amino group in the monoclinic anhydrate is hydrogen bonded to the ionized carboxyl groups of two molecules, while the amino group of the orthorhombic trihydrate is hydrogen bonded to a single carboxylate group and to the waters of hydration that link other molecules in the structure. The powder patterns of these two materials are shown in Fig. 3 and are seen to be readily distinguishable from each other. Amoxycillin trihydrate has been found to crystallize in the same space group as does ampicillin trihydrate, and it exhibits a very similar pattern of hydrogen bonding [21]. However, the dimensions of the two unit cells differ significantly, and this fact is

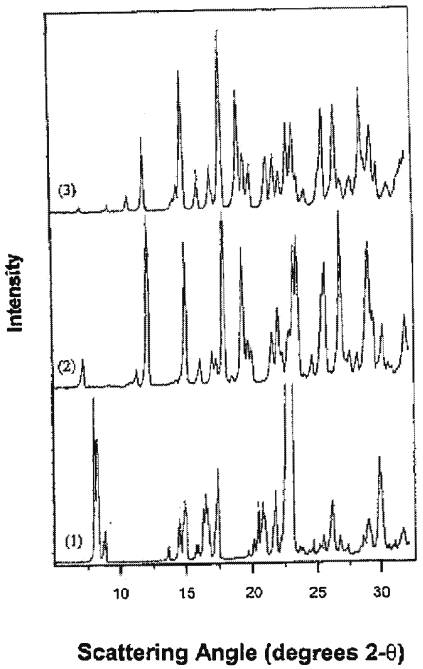

Fig. 3 X-ray powder diffraction patterns of (1) ampicillin anhydrate, (2) ampicillin trihydrate, and (3) amoxicillin trihydrate.

reflected in the differences among the relative intensities of the corresponding peaks contained in Fig. 3. Even though the two structures would be considered as being isostructural, the XRPD patterns of the two trihydrate phases readily permit an unambiguous identification and distinction between these.

X-ray powder diffraction can also be used for the quantitative determination of phase composition, and this approach has been discussed in detail [22]. In one particularly well-developed example, XRPD was used to quantitate the relative amounts of the anhydrate and dihydrate phases existing in carbamazepine samples [23]. The method was based on the observation that the XRPD of each phase

featured a scattering peak unique to each form, which was noted at a scattering angle where no scattering was observed for the other phase. Unlike loose powders, compressed samples yielded highly reproducible intensity values, so pelletized materials were used for the data acquisition. Good correlation between sample composition and scattering intensities was obtained in standard materials, permitting the generation of analytical relations suitable for the analysis of analyte samples.

The degree of crystallinity associated with a sample can often be established using powder x-ray diffraction. If the patterns of 100% crystalline and 100% amorphous material can be established, then the integrated peak intensity of the analyte is used to deduce the percent crystallinity. Such methodology has been used to measure the crystallinity of digoxin [24] and calcium gluceptate [25]. The XRPD method is extremely important during the characterization of lyophilized materials, since the stability of a crystalline solid is expected to exceed that of an amorphous or disordered solid. For instance, the technique has been used to study the properties of lyophilized imipenem [26].

III. MORPHOLOGY: MICROSCOPY

An extremely important tool for the characterization of polymorphs and solvates is that of microscopy, since the observable habits of differing crystal structures must necessarily be different and therefore useful for the characterization of such systems [27]. Common sense would dictate that the visual observation of such materials would immediately follow an x-ray crystallographic study, which would in principle make the science of optical crystallography [28–30] an essential aspect of any program of study. A review of crystallography from the pharmaceutical viewpoint is available [31].

As stated in an earlier section, a crystal is a polyhedral solid, bounded by a number of planar faces that are normally identified using the Miller indices. The arrangement of these faces is termed the *habit* of the crystal, and the crystal is built up through the repetition of the unit cell. The three-dimensional basic pattern of molecules in a solid

form the space lattice, and the application of simple geometry has shown that only 14 different types of simple space lattices are possible. By taking combinations of the various lattices possible for each crystallographic system, it has also been determined that all solids must belong to one of 230 space groups [28–30].

Both optical and electron microscopies have found widespread use for the characterization of polymorphs and solvates. Although optical microscopy is more limited in the range of magnification suitable for routine work (working beyond 600× being difficult when observing microcrystalline materials), the use of polarizing optics introduces enormous power into the technique not available with other methods. Electron microscopy work can be performed at extraordinarily high magnification levels (up to 90,000× on most units), and the images that can be obtained contain a considerable degree of three-dimensional information. The two methods are complementary in that each can provide information not obtainable by the other. With judicious use of these techniques, one can obtain substantial characterization of a polymorphic system. These data are extremely useful during the early stages of drug development, since often only a limited amount of the drug candidate is available at that time.

The literature pertaining to microscopy and its applications is quite large but fortunately is updated periodically [32]. A number of texts and review articles have been written that cover light or electron microscopy or some combination of the two [33,34]. It is beyond the scope of this chapter to provide a mechanical description of the various instruments; readers are referred to representative references for light microscopy [35–40] and electron microscopy [41–44].

McCrone has provided an excellent discussion of the synergistic aspects of optical and electron microscopies [45]. He concludes that electron microscopy yields excellent topographic and shape information and is most useful in forensic situations involving trace evidence characterization and identification. When polarizing optics are used during a light microscopy study, the optical properties of the crystals under investigation can also be determined. This latter aspect is extremely useful in the characterization of polymorphs and solvates, and consequently polarizing optical microscopy is an extremely important tool for the study of such systems.

used for the characterization of materials that evolve corrosive gases during the heating process. DTA analysis is highly useful as a means for the determination of compound melting points, although in systems capable of undergoing phase changes the analyst must always be aware of such concerns.

Methodology appropriate for the measuring of DTA profiles has been extensively reviewed [71,72] and need only be outlined here. Both the sample and the reference materials are contained within the same furnace, whose temperature program is externally controlled. The outputs of the sensing thermocouples are amplified, electronically subtracted, and finally shown on a suitable display device. If the observed change in enthalpy ΔH is positive as in the case of endothermic reactions, the temperature of the sample will lag behind that of the reference. If the ΔH is negative (exothermic reaction), the temperature of the sample will exceed that of the reference.

Wendlandt has provided an extensive compilation of conditions and requirements that influence the shape of DTA thermograms [73]. These can be divided into instrumental factors (furnace atmosphere, furnace geometry, sample holder material and geometry, thermocouple details, heating rate, and thermocouple location in the sample) and sample characteristics (particle size, thermal conductivity, heat capacity, packing density, swelling or shrinkage of sample, mass of sample taken, degree of crystallinity). A sufficient number of these factors are under the control of the operator, thus permitting selectivity in the methods of data collection. The ability to correlate an experimental DTA thermogram with a theoretical interpretation is profoundly affected by the details of heat transfer between the sample and the calorimeter [74].

The calibration of DTA systems is dependent on the use of appropriate reference materials rather than on the application of electrical heating methods. The temperature calibration is normally accomplished with the thermogram being obtained at the heating rate normally used for analysis [75], and the temperatures known for the thermal events are used to set temperatures for the empirically observed features. Recommended reference materials that span melting ranges of pharmaceutical interest include benzoic acid (melting point 122.4°C), indium (156.4°C), and tin (231.9°C).

The simplest and most straightforward application of DTA analysis is concerned with studies of the relative stability of polymorphic forms. For example, DTA thermograms enabled the deduction that one commercially available form of chloroquine diphosphate was phase pure while another consisted of a mixture of two polymorphs [76]. DTA analysis was used to demonstrate that even though different crystal habits of sulfamethazine could be obtained, these in fact consisted of the same anhydrous polymorph [77]. In a study aimed at profiling the dissolution behavior of the three polymorphs and five solvates of spironlactone, DTA analysis was used in conjunction with powder x-ray diffraction to establish the character of the various materials [78].

In one study, it was found that three different crystalline forms of phenylbutazone could be obtained by the spray-drying of methylene chloride solutions at different temperatures, and that neither of these corresponded to the stable form that was obtained at the highest drying temperatures [79]. In fact, one of these could not be obtained by any conventional crystallization process but was only detected under conditions involving a slower solvent evaporation rate. As shown in Fig. 6, the DTA thermograms of each form are distinct and permit a facile distinction among the isolated forms.

Prior to the widespread use of differential scanning calorimetry, DTA analysis was the only method whereby one could obtain heats of transition or fusion. For example, sulfathiazone was found to undergo a transition from Form I to Form II at 161°C, for which the heat of transition was determined to be 1420 cal/mol [80]. The heat of fusion directly obtained for Form II was found to be 5970 cal/mol, which compared favorably with the heat of fusion determined for the material resulting from the conversion of Form I (5960 cal/mol). In another study, heats of fusion were determined for sixteen sulfonamides, some of which exhibited polymorphism and some of which did not [81]. In this work, a fuller understanding of the thermodynamics was provided, where the entropies as well as the enthalpies of the various processes were deduced.

C. Differential Scanning Calorimetry

In many respects, differential scanning calorimetry (DSC) is similar to the DTA method, and analogous information about the same type of thermal events can be obtained. However, DSC is far easier to use

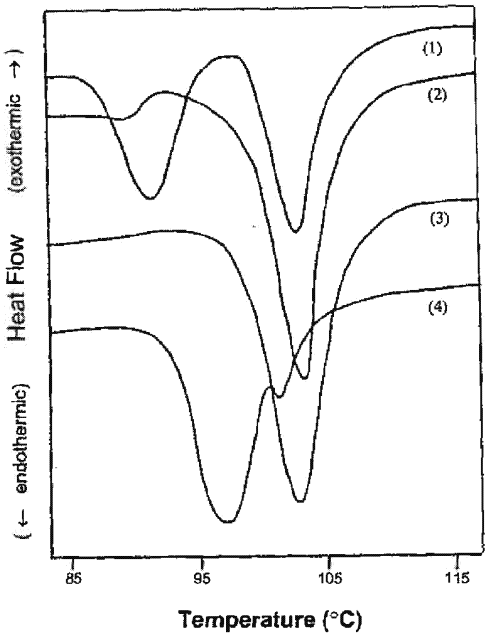

Fig. 6 Differential scanning calorimetry study of various spray-dried forms of phenylbutazone. Shown are the thermograms of (1) Form α, (2) Form β, (3) Form δ, and (4) Form ε. (The figure was adapted from data contained in Ref. 79.)

routinely on a quantitative basis, and for this reason it has become the most widely accepted method of thermal analysis for the pharmaceutical industry. The relevance of the DSC technique as a tool for pharmaceutical scientists has been amply discussed in numerous reviews [66,83–86], and a general chapter on DSC is documented in the *United States Pharmacopoeia* [87].

In the DSC method, the sample and reference materials are maintained at the same temperature, and the heat flow required to keep the equality in temperature is measured. DSC plots are therefore obtained as the differential rate of heating (in units of W/s, cal/s, or J/s) against temperature [88]. The area under a DSC peak is directly proportional to the heat absorbed or evolved by the thermal event, and integration

of these peak areas yields the heat of reaction (in units of cal/s · g or J/s · g).

Two types of DSC measurements are possible, which are usually identified as power-compensation DSC and heat-flux DSC, and the details of each have been fully described [88,89]. In power-compensated DSC, the sample and reference materials are kept at the same temperature by the use of individualized heating elements, and the observable parameter recorded is the difference in power inputs to the two heaters. In heat-flux DSC, one simply monitors the heat differential between the sample and the reference materials, with the methodology not being much different from that used for DTA.

In the DTA measurement, an exothermic reaction is plotted as a positive thermal event, while an endothermic reaction is usually displayed as a negative event. Unfortunately, the use of power-compensation DSC results in endothermic reactions being displayed as positive events, a situation counter to the latest IUPAC recommendations [90]. When the heat-flux method is used to detect the thermal phenomena, the signs of the DSC events concur with those obtained using DTA and also agree with the IUPAC recommendations.

The calibration of DSC instruments is normally accomplished through the use of compounds having accurately known transition temperatures and heats of fusion. A list of the standards currently supplied by the National Technical Information Service (NTIS) [91] is provided in Table 2. Once the DSC system is properly calibrated, it is trivial to obtain the melting point and enthalpy of fusion data for any compound upon integration of its empirically determined endotherm and application of the calibration parameters. The current state of methodology is such, however, that unless a determination is repeated a large number of times, the deduced enthalpies must be regarded as being accurate only to within approximately 5%.

As has been noted for DTA analysis, differential scanning calorimetry can also be used to establish the melting points of polymorphic species. For example, gepirone hydrochloride has been obtained in three polymorphic forms, which were found to melt at 180°C, 200°C, and 212°C [92]. In this work, it was shown that Forms I and II, and Forms I and III, were enantiotropic pair systems, but that Forms II and III were monotropic with respect to each other. The two polymorphs of phenylephrine oxazolidine each exhibited well-defined melting

points and could easily be distinguished on the basis of their thermograms [93].

Some of the most interesting DSC studies have been conducted on metastable phases that undergo a phase transformation to a more stable phase during the lifetime of the thermal analysis experiment. It was found that Form I of iopanoic acid yielded a single melting endotherm at 154°C, but that the thermogram obtained on Form II was much more complicated [94]. As shown in Fig. 7, Form II exhibits one endotherm at 133°C (the melting transition of Form I), an exotherm at 141°C (crystallization to Form II), and another endotherm at 153°C (melting of the recrystallized Form II). The literature abounds with similar examples where metastable polymorphs have been observed to convert

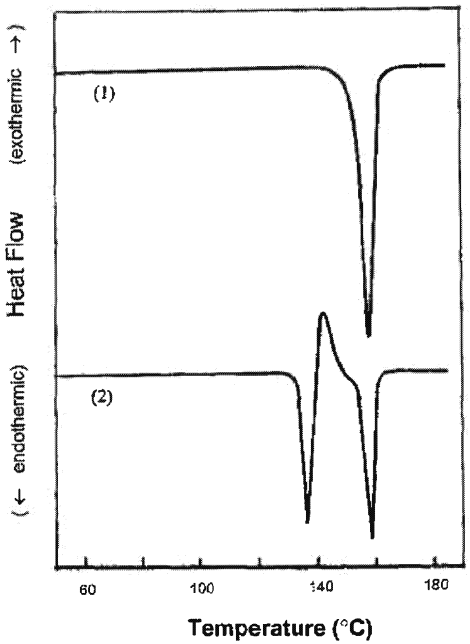

Fig. 7 Differential scanning calorimetry thermograms obtained for the (1) Form I and (2) Form II polymorphs of iopanoic acid. (The figure was adapted from data contained in Ref. 94.)

into more stable forms, with carbamazepine [95], piroxicam [96], and pireanide [97] being quoted as recent examples of work in this genre.

When studying the stability relationships between different polymorphic forms, the use of temperature cycling experiments has often proven to be very useful. Samples are heated to a preset temperature (but not high enough to induce a thermal decomposition event), cooled back to a lower temperature, and then reheated. Alterations in the recorded thermograms resulting from the cycling process can provide information about the ease of phase conversion. For example, such work enabled deductions to be made regarding the relative stabilities of the polymorphs of 1,2-dihydro-6-neopentyl-2-oxonicotinic acid [98]. The polymorphism associated with glyceryl monostearate was found to exert a profound effect upon the stability of formulations containing raw materials obtained from different sources, but the system could be understood in terms of phase transformations brought about by repeated melting and congealing cycles [99]. In fact, the use of cycling experiments may be essential to eliminate artifacts from entering into the construction of phase diagrams [100].

DSC techniques may be used to determine the kinetics of solid-state transformations as well. The kinetics associated with the transformation of disopyramide Form I to Form II were followed by changes in the DSC thermograms, and an activation energy of 144 kJ/mol was calculated for the system [101]. According to this work, the model proposed by Prout and Tompkins [102] for decomposition in solid phases (with no prior melting or liquefaction) appears to be appropriate for solid-state polymorphic transitions. As anticipated for any solid-state reaction, the phase change would initiate at defect sites, producing energetically favorable configurations that would serve as templates for continued phase transformation. In a similar work, a combination of DSC and XRPD studies was used to evaluate the kinetics associated with the various polymorphic transitions of phenylbutazone [103].

DSC analysis is often used in conjunction with structural techniques during the characterization of hydrate and solvate systems, with the thermal method being used to pinpoint the transition temperature range over which the bound water or solvent can be liberated. For instance, although a number of solvate forms could be crystallized for ethynylestradiol, the different solvent molecules were found incapable of exerting any effect on the conformation of the drug [104]. DSC

studies permitted the deduction of the relative stabilities of the three solvates of alprazolam [105] and contributed to the characterization of the solvate systems identified for norfloxacin [106] and dehydroepiandrosterone [107].

The character of the anhydrous phase resulting after the desolvation process has taken place can be effectively understood using DSC analysis. In many cases, loss of solvation or hydration molecules leads to the formation of an amorphous material, but this is not always the case. It was established that the acetone, isopropanol, and ethyl acetate solvates of cyclopenthiazide all reverted to Form I (a known anhydrous phase) above the endotherm corresponding to loss of solvent [108]. In other systems, loss of solvation results in the formation of a metastable phase, which undergoes a phase conversion to the stable anhydrate phase before overall melting takes place. Such behavior is illustrated in Fig. 8, where it was reported that the *tert*-butanol and dioxane solvates of piretanide exhibited a recrystallization after desolvation, but that the propylene glycol and dimethylformamide solvates did not [109].

V. MOLECULAR MOTION: VIBRATIONAL SPECTROSCOPY

The energies associated with the vibrational modes of a chemical compound lie within the range of 400–4000 cm^{-1}. These modes can be observed directly through their absorbance in the infrared region of the spectrum, or through the observation of the low-energy scattered bands that accompany the passage of an intense beam of light through the sample (the Raman effect). In either case, the use of Fourier-transform methodology has vastly improved the quality of data that can be obtained [110]. Most workers are familiar with the use of mid-infrared spectra for identity purposes, where the pattern of absorption bands is taken to be diagnostic for a given compound. However, it has come to be recognized that the vibrational spectra of solid materials will reflect details of the crystal structure, and hence these methods can be used in the spectroscopic investigation of polymorphs and solvates [111,112].

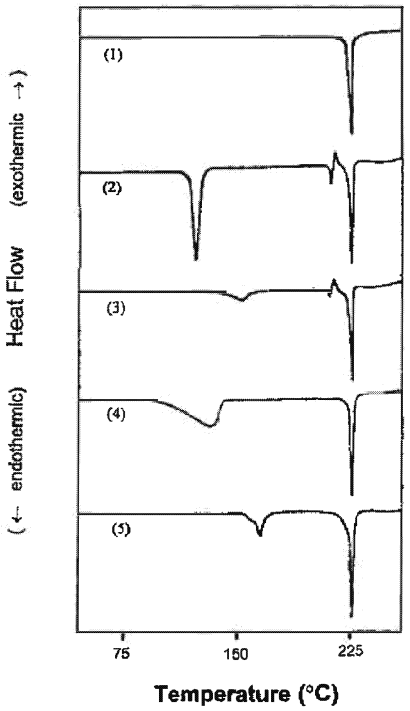

Fig. 8 Differential scanning calorimetry study of various solvates of piretanide. Shown are the thermograms of (1) the anhydrate phase, (2) the *tert*-butanol solvate, (3) the dioxane solvate, (4) the propylene glycol solvate, and (5) the dimehylformamide solvate. (The figure was adapted from data contained in Ref. 109.)

In the best-designed studies of polymorphic or solvate systems, the purpose of the vibrational spectroscopy investigation should be to gather information from the observed pattern of vibrational frequencies and to use these data to understand the structural aspects that yield crystallographic differences. Once suitable spectral features are identified from this work, they can be used to develop easily performed methods for the quantitative analysis of one polymorph (or solvate) in the presence of the other. Unfortunately, all too many workers are merely